27. (New) The kit of claim 26, wherein the kit further comprises a restriction endonuclease which recognizes a sequence comprising a residue in human dihydropyrimidine dehydrogenase genomic DNA at the position indicated as nucleotide 434 of SEQ ID NO: 1.

a3 cont.

28. (New) The kit of claim 27, wherein the restriction endonuclease recognizes a Mae II cleavage site.

IN THE ABSTRACT:

Please insert the abstract provided herein on a separate sheet.

REMARKS

With this Amendment, claims 1-11, 15-17, and 20-28 are pending. The specification has been amended to list the priority data for the application. The specification has also been amended such that Figures 1A and 1B are listed in the Decription of the Drawings, rather than Figure 1. An Abstract, essentially identical to the one filed with the original priority application, has been provided. No new matter has been added.

The claims have been amended to recite a particular splicing polymorphism at a defined position in the DPD gene, identified by reference to a nucleotide position in SEQ ID NO: 1. It is contemplated that reference to this position in SEQ ID NO: 1, also referenced as position 1987 of the DPD gene, should encompass any residue found in the equivalent position in all DNAs originating from the human DPD locus at the disclosed intron-exon boundary. Support for explicit recitation of this polymorphism may be found, inter alia, at page 14, lines 6-8, page 22, lines 22-25, and Figures 2-4 of the specification.

The claims have also been amended to recite human genomic DNAs and human DPD genes. Support for the amendment may be found, inter alia, at page 21, line 23, and page 23, line 30, of the specification.

The claims have also been amended to recite amplification primers which bind to DNA sequences within 500 nucleotides or 100 nucleotides of the polymorphism at the exon-intron boundary. Support for the amendment may be found, inter alia, at page 15, lines 17-18 of the specification.

The claims have also been amended to clarify that restriction endonucleases other than those that recognize Mae II sites, may be employed in practice of the invention. The general use of restriction endonucleases that recognize sites created or destroyed by DPD polymorphisms may be found at page 16, lines 14-29 of the specification.

The claims have also been amended for grammatical clarity and to correct typographical errors.

Finally, new claims 20-28 parallel existing claims as originally filed or as modified by this Amendment. No new matter is added.

Applicants appreciate the Examiner's careful analysis. The Examiner's objections and rejections are addressed in the order they were made in the April 18, 2001 Office Action.

I. The invention

The invention relates to the discovery that some humans are deficient in expression of the enzyme dihydropyrimidine dehydrogenase (DPD) due to a splicing mutation at the exon-intron boundary for the exon encoding amino acids 581-635 of human DPD. Since DPD is one of the enzymes that metabolize the chemotherapeutic drug 5-fluorouracil, deficiencies in DPD expression may result in acute toxicity in patients being treated with 5-fluorouracil. By screening prospective patients for the splicing mutation, a large proportion of 5-fluorouracil-sensitive patients may be identified prior to commencing chemotherapy.

II. Objections to the Specification

The specification was objected to for informalities in the Brief Description of the Drawings, and the omission of SEQ ID NOs in reference to particular primers. Applicants have amended the Brief Description of the Drawings to explicitly list filed Figures 1A and 1B, and to insert the proper SEQ ID NOs in reference to the primers. Applicants therefore respectfully request that the objection be withdrawn.

The specification was further objected to for omission of a listing of the priority documents for the application. Applicants have amended the specification to list the priority documents claimed upon filing, and respectfully request that the objection be withdrawn.

The specification was further objected to for omission of an Abstract of the Disclosure. Applicants have provided an Abstract herein, and respectfully request that the objection be withdrawn.

Claims 8 and 10 were objected to for grammatical errors. Applicants have amended claims 8 and 10 to be grammatically correct, and respectfully request that the rejection be withdrawn.

III. Rejections under 35 U.S.C. § 112, 2nd paragraph (indefiniteness)

Claims 1-6 were rejected as indefinite, in that the scope of nucleotides included in the term "intron-exon boundary" was allegedly unclear. The pending claims now refer to particular nucleotide positions defined by reference to SEQ ID NO: 1, rather than the "intron-exon boundary". Applicants therefore respectfully request that the rejection be withdrawn.

Likewise, claims 2 and 7, which were rejected as allegedly indefinite for recitation of the term "the region flanking the exon which encodes amino acids 581-635," now refer to particular nucleotide positions defined by reference to SEQ ID NO: 1 rather than the allegedly indefinite terminology. Applicants therefore respectfully request that the rejection be withdrawn.

Claims 3, 4, 8, 9, 13, 14, and 17-19 were rejected as not compliant with the sequence rules for failure to list the SEQ ID NO of primer DELF1 and DELR1. The pending claims no longer recite these primers explicitly, and Applicants respectfully request that the rejection be withdrawn.

Claims 3, 8, 13, 14, 18, and 19 were rejected for the alleged indefiniteness of the term "stringent conditions." The term "stringent conditions" no longer appears in the pending claims, and Applicants respectfully request that the rejection be withdrawn.

Claims 18 and 19 were rejected for recitation of "the composition" without antecedent basis. Amended claim 19 now recites "the kit" of the preamble in the claim body, and Applicants respectfully request that the rejection be withdrawn.

IV. Rejection under 35 U.S.C. § 112, first pargraph (written description)

Claims 1-19 were rejected under 35 U.S.C. § 112, first paragraph, for alleged lack of written description. According to the rejection, only a single representative DPD gene, a single representative splicing defect, and a single representative species of PCR primers were disclosed.

The rejection therefore argued that these single species were insufficient to describe the genera claimed by claims 1-19. To the exent that the rejection still applies to the amended claims, Applicants respectfully traverse.

As amended, the pending claims recite only the human DPD gene, and a splicing defect characterized by a mutation at position 434 of SEQ ID NO: 1. Applicants have disclosed the relevant sequences of the human DPD gene and the nature of mutations at position 434, and therefore submit that the amended claims are fully supported by the disclosure of the specification.

Several of the amended claims do claim a genus of amplification primers that may be used to amplify human genomic DNA comprising position 434 of SEQ ID NO: 1 (position 1987 of the DPD gene). The specification discloses the following structural and functional properties of this genus:

(1) The primers bracket the disclosed polymorphism in the DPD gene.

(2) The primers are of suitable length for PCR.

(3) The primers bind to human genomic DNA near the DPD locus with adequate specificity.

(4) The binding sites of the primers are near enough to the polymorphism to support a PCR reaction, i.e., less than about 10 kb from the polymorphism, or, in some embodiments, less than 500 or 100 nucleotides from the polymorphism.

(5) Three species of the genus are disclosed (DELF1, DELR1, and DPD15).

The specification further discloses intronic DNA sequences suitable as primer binding sites; exon DNA sequences also suitable as primer binding sites were known from the prior art. Knowledge of the primer binding sequence necessarily provides knowledge of the primer sequence. Thus, Applicants have disclosed a combination of both structural and functional characteristics that are common to the claimed primers and distinguish them from all other primers. Variability in the genus is constrained by the requirements that the primers recognize sites in disclosed or known genomic DNA, and that the primers are of appropriate length and distance from the polymorphism to support amplification. Given the ordinary skill of those in the art in designing and selecting amplification primers, the combination of structural and functional characteristics disclosed by Applicants would allow one of skill in the art to visualize a sufficient number of primers so as to

conclude that Applicant was in possession of the necessary common attributes psosessed by the genus. Applicants therefore respectfully request that the rejection be withdrawn.

V. Rejection under 35 U.S.C. § 112, first paragraph (enablement)

Claims 1-19 were rejected under 35 U.S.C. § 112, first paragraph, for alleged lack of enablement. According to the rejection, the disclosure of the specification could not support the scope of the claims, since (A) not all genomic DPD DNA sequences from other organisms have been characterized, (B) not all DPD genes are completely homologous, (C) one could not design primers that would hybridize to any genomic or intronic DPD DNA, (D) not all mutations in an intron-exon boundary of the DPD gene might lead to a splicing defect, and (E) the specification failed to provide guidance as to which choice of primers or mutations would lead to success in identifying 5-fluorouracil sensitivity. To the extent the rejection applies to the amended claims, Applicants respectfully traverse.

As amended, the pending claims recite only the human DPD gene, and only a splicing defect caused by mutation of the residue at position 1987 of the human DPD gene. Detection of this polymorphism in human DPD DNA is explicitly taught by the specification, and the Examiner has recognized that the general method of detecting splicing defects caused by this polymorphism is enabled (paragraph 14 of the Office Action).

Design of amplification primers for the methods and compositions of the invention would be a matter of routine experimentation for the skilled artisan. As noted above in connection with the written description requirement, the specification provides multiple characteristics of the claimed primers that would guide the skilled artisan in their design. Once both the mutated residue and the sequence of the surrounding DNA are known, the design of primers and their use in amplifying the mutated residue may be accomplished by routine experimentation, e.g., optimizing the sequence of the primers and the conditions for amplification. Likewise, multiple methods for detecting mutations at specified positions in sequenced DNA are known to those of skill in the art, and could be employed to identify individuals with mutations at position 1987 of the human DPD gene. Applicants therefore submit that one of skill in the art could pratice the claimed invention without undue experimentation, and respectfully request that the rejection be withdrawn.

VI. Rejection under 35 U.S.C. § 103

Claims 1-3, 6-8, 10-12, 15, and 16 were rejected under 35 U.S.C. § 103(a) as allegedly obvious in light of Gonzalez et al. (Trends Pharmacol Sci 16:325-327) and Meinsma et al. (DNA Cell Biol 14:1-6). According to the rejection, Gonzalez discloses that certain patients exhibiting DPD deficiency have a deletion of 165 bp complete exon in their mRNA, while Meinsma discloses that the 165 bp exon is skipped as the result of a mutation causing defective splicing. To the extent that the rejection still applies to the amended claims, Applicants traverse.

Amended claims 1-9 and 16-17, as well as new claims 20-23 and 25-28, all recite detection of a particular polymorphism at position 1987 of the DPD gene. While Gonzalez and Meinsma suggest that some sort of splicing mutation leads to skipping of the 165 bp exon, they do not indicate that the mutation in question is a point mutation, nor that it must be at the intron-exon boundary, nor that it resides at position 1987 of the human DPD gene. Moreover, even if one of skill in the art would somehow be motivated to sequence position 1987 nonetheless, these references would not provide a reasonable expectation of success for the reasons set forth below.

In vertebrates, several features of the pre-mRNA are required for accurate splicing. These include the sequences at the intron-exon boundary, the presence of an invariant adenosine residue at the branch point, and a pyrimidine-rich region near the 3' end of the intron. Mutations in any of these elements can lead to splicing failure and exon skipping. Based on the information in Meinsma and Gonzales, one of skill in the art would not know if exon skipping was due to mutations at the intron/exon boundaries, or at the branch point, or in the pyrimidine tract, or even at more distant positions. While Gonzales discloses that the intron/exon boundaries were being sequenced, this teaching alone - in the absence of actual sequence - would not lead the skilled artisan to conclude that exon skipping was due to any mutations at the intron/exon boundaries. Hence, the skilled artisan would not have a reasonable expectation of success in identifying non-functional DPD alleles by detecting mutations at the boundaries of the 165 bp exon - much less in determining the sequence of the nucleotide at position 1987.

Furthermore, even of one of skill in the art were motivated to sequence position 1987 in the DPD gene, one would need to know the sequence of the surrounding intronic DNA in order to determine the presence or absence of a G as this position. As neither Meinsma nor Gonzalez provide this information, they are not enabling references.

As noted above, Meinsma teaches that the 165 bp exon is skipped in some DPD deficient patients. However, Meinsma does not teach sequencing the boundaries of the 165 bp exon to determine if this exon has wild-type intron/exon boundaries. According to Meinsma, only the 3' boundary of the preceding exon had been identified (see p. 4, col. 1). Neither the 5' nor the 3' end of the 165 bp exon was known. In fact, Meinsma specifically says that no genomic clone of the 165 bp exon had been isolated (p. 4, col. 1, lines 5-7).

The skilled artisan understands that determining the sequence of a nucleotide in genomic DNA requires isolation of genomic DNA containing the nucleotide in question, and some knowledge of the surrounding DNA sequence. Although Gonzalez discloses that sequencing the intron-exon boundaries was underway, Gonzalez does not disclose any intronic sequence. Even if a general method of isolating intronic DNA based on the known exon sequence was known, the specific sequence of the intronic DNA could not be obvious in the absence of other prior art suggesting the sequence of the intronic DNA (see *In re Deuel*, 51 F.3d 1552 at 1559, 34 USPQ2d 1210 at 1215 (Fed. Cir. 1995)). Without either a genomic clone, or the sequence of the introns flanking the 165 bp exon, the skilled artisan would have no way to determine the sequence of the nucleotide at position 1987 of the DPD gene (residue 434 of SEQ ID NO: 1). As such, the skilled artisan could not detect any splicing defects in the DPD gene by determining whether a G residue was present at position 1987 of the human DPD gene. Therefore, the Meinsma and Gonzalez references cannot provide an enabling disclosure that would render claims 1-9, 16-17, 20-23 and 25-28 obvious. Applicants therefore respectfully request that the rejection be withdrawn.

Amended claims 10, 11 and 15 and new claim 24 do not recite determining the sequence of the nucleotide at position 1987 of the human DPD gene. However, they recite primers which hybridize to human DPD intronic genomic DNA. In order to create primers hybridizing to human DPD intronic genomic DNA, the skilled artisan would need to know the sequence of the intronic DNA. As discussed above, the intronic sequence of the DPD gene is not disclosed by Meinsma or Gonzalez, nor would the intronic sequence be obvious in light of Meinsma or Gonzales. Therefore, neither Meinsma nor Gonzales provide a disclosure that would enable one of ordinary skill in the art to make the primers of claims 10, 11, 15, or 24. Since Meinsma or Gonzales cannot therefore render claims 10, 11, 15 or 24 obvious, Applicants respectfully request that the rejection be withdrawn.

VII. Sequence Listing

This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-7, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

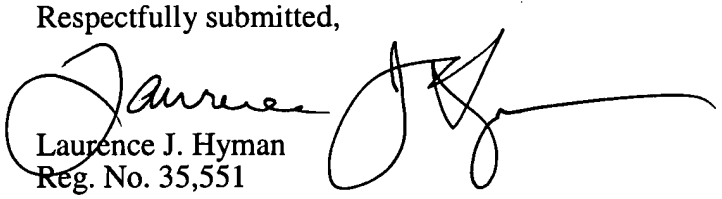
The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



Laurence J. Hyman
Reg. No. 35,551

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
KAW/JL1/jhd
SF 1251780 v1

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The paragraph beginning at 5, line 8 has been amended as follows:

Figures 1A and 1B [1] provide[s] genomic sequence (SEQ ID NO:1) from the DPD gene in the region of a splicing mutant which leads to the loss of an exon in the mRNA which encodes amino acids 581-635 (SEQ ID NO:2). The primer binding sites for DELF-1 (SEQ ID NO:3) and DELR1 (SEQ ID NO:4) are indicated. The primer binding site for DPD15F (SEQ ID NO:5) and DPD15R (SEQ ID NO:6) (*see*, Meinsma *et al.* (1995) *DNA and Cell Biology* 14(1): 1-6) are also indicated. The Mae II site at the 3' splice junction is indicated. In a mutant form, the G nucleotide at the 3' splice junction (residue 434) is mutated to an A nucleotide.

Figure 2 provides further details of PCR reaction components for amplifying the region of a splicing site mutant (SEQ ID NO:7) The exon is underlined. The splice site which is polymorphic is in bold text. Primers DELF1 and DELR1 are indicated (SEQ ID NOS:3 and 4)

The paragraph beginning at page 21, line 23, has been amended as follows:

The PCR primers were selected so as to bracket the exon that is not present in the DPDD gene and part of the two introns on either side of this exon. Primers were synthesized using an Applied Biosystems 394 DNA & RNA synthesizer. The forward primer was DELF1, which encompassed nucleotide 154 to nucleotide 175 of the DP[F]DD gene sequence and had the sequence TGCAAATATGTGAGGAGGGACC (SEQ ID NO: 3) (*see also* Figure 1 and Figure 2). The reverse primer was DELR1, which encompassed nucleotide 563 to nucleotide 542 of the DP[F]DD gene sequence and had the sequence CAAAGCAACTGGCAGATTC (SEQ ID NO: 4) (*see also* Figure 1 and Figure 2). PCR was carried out in 50 µl of a reaction mixture consisting of 10 m*M* Tris-HCl pH 8.3, 50 m*M* KCl, 2.5 m*M* $MgCl_2$, 0.5 m*M* dNTPs, 1 µ*M* of

each primer, and 2.5 units *Taq* polymerase (Roche Molecular Systems) for 30 cycles denaturing at 96°C for 1 min, annealing at 55°C for 1 min, and extending at 72°C for 2 min. The amplified products were extracted with 1 volume of chloroform and purified by filtration through Centricon 100 filter units.

The paragraph beginning at page 23, line 29, has been amended as follows:

The DPD cDNA was used as a probe to isolate a P1 clone containing about 100 kbp of the human *DPD* gene (PAC 5945) from a high density PAC human genomic library (Genome Systems, St. Louis, MO). Southern blotting was used to confirm that the P1 clone contained the deleted exon using a probe located within the deleted fragment. This probe was synthesized from the DPD cDNA by using the primers: DPD15 (forward): 5' TTGTGACAAATGTTTCCC 3' (SEQ ID NO: 5) and DPD15R (reverse): 5' AGTCAGCCTTTAGTTCAGTGACAC 3' (SEQ ID NO: 6) to specifically amplify the putative exon. PCR conditions were as indicated below but extension was carried out at 72°C for 1 min. This PCR fragment was purified using a Wizard PCR purification kit (Promega, Madison, WI), labeled with [α^{32}P]-dCTP and hybridized with the clone PAC 5945. DNA was purified from this genomic clone using Qiagen columns (Qiagen, Chatsworth, CA), and the 5' and 3' end of the deleted exon and adjacent intronic regions were sequenced by chromosome walking from within the deleted exon using dideoxy terminator chemistry and an ABI 373A DNA sequencer (Applied Biosystems, Foster City, CA). The intronic sequences obtained allowed the selection of appropriate primers (*e.g.*, delF1 and delR1) to amplify from genomic DNA the complete exon and immediate flanking intronic sequences. All primers used in this study were synthesized with a 391 DNA&RNA synthesizer (Applied Biosystems, Foster City, CA).

The paragraph beginning at page 24, line 16, has been amended as follows:

A 409 bp PCR genomic fragment corresponding to the deleted exon (from G1822 to C1986 in Yokota, *et al.* (1994) *J. Biol. Chem.* 269: 23192-23196) plus the flanking intronic sequences containing the AG and GT splicing consensus sequences was amplified from human

genomic DNA using the primers delF1 (forward) 5' TGCAAATATGTGAGGAGGGACC 3' (SEQ ID NO: 3) and delR1 (reverse) 5' CAGCAAAGCAACTGGCAGATTC 3' (SEQ ID NO: 4). PCR amplification was carried out in a 100 µl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 µM of each primer, 2.5 units of *Taq* Polymerase (Roche Molecular Systems) and 500 ng of genomic DNA template for 31 cycles by denaturing at 94°C for 1 min, annealing at 60°C for 1 min and extending at 72°C for 2 min. Subjects identified as wild type, heterozygous or homozygous for the splicing mutation could be distinguished by digesting the PCR product with the restriction endonuclease Mae II (Boehringer, Mannheim, Indianapolis, IN) and electrophoresis in 1% regular, 3% NuSieve agarose gels (FMC Bioproducts, Rockland, ME). The genotypes obtained were verified by sequencing the 409 bp PCR product. The sources of the genomic DNA samples for the different ethnic groups correspond to those previously described (Fernandez-Salguero *et al.* (1995) *Am. J. Hum. Genet.* 57: 651-660).

IN THE CLAIMS:

Claims 12, 13, 14, 18, and 19 have been cancelled. Claims 1-11 and 15-17 have been amended as follows. New claims 20-28 have been added as follows.

1. (Amended) A method of detecting a splicing defect in a human dihydropyrimidine dehydrogenase gene, comprising determining whether a human genomic DNA encoding the human dihydropyrimidine dehydrogenase gene [has a wild-type intron-exon boundary for an exon which encodes amino acids 581-635 of a corresponding wild-type dihydropyrimidine dehydrogenase protein] comprises a G residue at the position indicated as nucleotide 434 of SEQ ID NO: 1, wherein substitution of the G residue with an A residue causes a splicing defect in the human dihydropryimidine dehydrogenase gene.

2. (Amended) The method of claim 1, wherein the method comprises the step of amplifying human intronic dihydropyrimidine dehydrogenase genomic DNA [encoding the dihydropyrimidine dehydrogenase in the region flanking the exon which encodes amino acids

581-635] which comprises a residue at the position indicated as nucleotide 434 of SEQ ID NO: 1.

3. (Amended) The method of claim 2, wherein the method comprises amplifying the genomic DNA with a primer which hybridizes to a [dihydropyrimidine dehydrogenase intronic nucleic acid which hybridizes to a primer selected from the group of primers consisting of DELF1, and DELR1 under stringent conditions] human dihydropyrimidine dehydrogenase genomic nucleotide sequence located within 500 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1.

4. (Amended) The method of claim 2, wherein [DNA amplified with the primer is cleaved with a restriction endonuclease which recognizes a Mae II cleavage site] the presence or absence of the G residue is detected by digestion of the amplified DNA with a restriction endonuclease.

5. (Amended) The method of claim 1, wherein the [sequence of the intron-exon boundary] presence or absence of the G residue is detected by [is determined] using an oligonucleotide array.

6. (Amended) A method of screening human patients for sensitivity to 5-fluorour[i]acil, comprising isolating a genomic DNA from the patient [which encodes the dihydropyrimidine dehydrogenase gene] and determining whether the [gene has a wild-type intron-exon boundary for an exon which encodes amino acids 581-635 of a corresponding wild-type dihydropyrimidine dehydrogenase protein] genomic DNA comprises a G residue at the position indicated as nucleotide 434 of SEQ ID NO: 1.

7. (Amended) The method of claim 6, wherein the method comprises the step of amplifying human intronic dihydropyrimidine dehydrogenase genomic DNA from the patient [encoding the dihydropyrimidine dehydrogenase in the region flanking the exon which encodes amino acids 581-635] which comprises a residue at the position indicated as nucleotide 434 of SEQ ID NO: 1.

8. (Amended) The method of claim 7, wherein the method comprises amplifying the genomic DNA with a primer which hybridizes to a [dihydropyrimidine dehydrogenase intronic nucleic acid which hybridizes to a primer selected from the group of primers consisting of DELF1 or DELR1 under stringent conditions] human dihydropyrimidine dehydrogenase genomic nucleotide sequence located within 500 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1.

9. (Amended) The method of claim 7, wherein [DNA amplified with the primers is cleaved with a restriction endonuclease which recognizes a Mae II cleavage site] the presence or absence of the G residue is detected by digestion of the amplified DNA with a restriction endonuclease.

10. (Amended) A composition comprising a [first] PCR primer which binds to a human dihydropyrimidine dehydrogenase intronic genomic nucleotide sequence located within 500 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1 [DNA 3' of a splice site in the genomic DNA for dihydropyrimidine dehydrogenase gene for an exon encoding amino acids 581-635, and a second PCR primer which binds to DNA 5' of a splice site in the genomic DNA for dihydropyrimidine dehydrogenase gene for an exon encoding amino acids 581-635].

11. (Amended) The composition of claim 10, wherein the [first] PCR primer binds to a human dihydropyrimidine dehydrogenase intronic genomic nucleotide sequence located within 100 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1 [intronic dihydropyrimidine dehydrogenase DNA].

15. (Amended) A kit comprising a container, a first PCR primer which binds to DNA 3' of a splice site in the human genomic DNA for [dihydropyrimidine dehydrogenase gene for] an exon encoding amino acids 581-635 of human dihydropyrimidine dehydrogenase, and a second PCR primer which binds to DNA 5' of a splice site in the human genomic DNA for [dihydropyrimidine dehydrogenase gene for] an exon encoding amino acids 581-635 of human dihydropyrimidine dehydrogenase, wherein at least one of the first or second PCR primers binds

to a human dihydropyrimidine dehydrogenase intronic genomic nucleotide sequence located within 500 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1.

16. (Amended) The kit of claim 15, wherein the kit further comprises instructions for the detection of [splicing site defects in the dihydropyrimidine dehydrogenase gene] the presence or absence of a G residue in human dihydropyrimidine dehydrogenase genomic DNA at the position indicated as nucleotide 434 of SEQ ID NO: 1.

17. (Amended) The kit of claim 15, wherein the kit further comprises [Mae II] a restriction endonuclease which recognizes a sequence comprising a residue in human dihydropyrimidine dehydrogenase genomic DNA at the position indicated as nucleotide 434 of SEQ ID NO: 1.

20. (New) The method of claim 2, wherein the method comprises amplifying the genomic DNA with a primer which hybridizes to a human dihydropyrimidine dehydrogenase genomic nucleotide sequence located within 100 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1.

21. (New) The method of claim 4, wherein the restriction endonuclease recognizes a Mae II cleavage site.

22. (New) The method of claim 8, wherein the method comprises amplifying the genomic DNA with a primer which hybridizes to a human dihydropyrimidine dehydrogenase genomic nucleotide sequence located within 100 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1.

23. (New) The method of claim 9, wherein the restriction endonuclease recognizes a Mae II cleavage site.

24. (New) The kit of claim 15, wherein at least one of the first or second PCR primers binds to a human dihydropyrimidine dehydrogenase intronic genomic nucleotide sequence located within 100 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1.

25. (New) The kit of claim 17, wherein the restriction endonuclease recognizes a Mae II cleavage site.

26. (New) A kit comprising a container, a first PCR primer which binds to DNA 3' of a splice site in the human genomic DNA for an exon encoding amino acids 581-635 of human dihydropyrimidine dehydrogenase, a second PCR primer which binds to DNA 5' of a splice site in the human genomic DNA for an exon encoding amino acids 581-635 of human dihydropyrimidine dehydrogenase, and instructions for the detection of the presence or absence of a G residue in human dihydropyrimidine dehydrogenase genomic DNA at the position indicated as nucleotide 434 of SEQ ID NO: 1.

27. (New) The kit of claim 26, wherein the kit further comprises a restriction endonuclease which recognizes a sequence comprising a residue in human dihydropyrimidine dehydrogenase genomic DNA at the position indicated as nucleotide 434 of SEQ ID NO: 1.

28. (New) The kit of claim 27, wherein the restriction endonuclease recognizes a Mae II cleavage site.

APPENDIX I
PENDING CLAIMS FOLLOWING ENTRY OF THIS AMENDMENT

1. (Amended) A method of detecting a splicing defect in a human dihydropyrimidine dehydrogenase gene, comprising determining whether a human genomic DNA encoding the human dihydropyrimidine dehydrogenase gene comprises a G residue at the position indicated as nucleotide 434 of SEQ ID NO: 1, wherein substitution of the G residue with an A residue causes a splicing defect in the human dihydropryimidine dehydrogenase gene.

2. (Amended) The method of claim 1, wherein the method comprises the step of amplifying human intronic dihydropyrimidine dehydrogenase genomic DNA which comprises a residue at the position indicated as nucleotide 434 of SEQ ID NO: 1.

3. (Amended) The method of claim 2, wherein the method comprises amplifying the genomic DNA with a primer which hybridizes to a human dihydropyrimidine dehydrogenase genomic nucleotide sequence located within 500 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1.

4. (Amended) The method of claim 2, wherein the presence or absence of the G residue is detected by digestion of the amplified DNA with a restriction endonuclease.

5. (Amended) The method of claim 1, wherein the presence or absence of the G residue is detected by using an oligonucleotide array.

6. (Amended) A method of screening human patients for sensitivity to 5-fluorouracil, comprising isolating a genomic DNA from the patient and determining whether the genomic DNA comprises a G residue at the position indicated as nucleotide 434 of SEQ ID NO: 1.

7. (Amended) The method of claim 6, wherein the method comprises the step of amplifying human intronic dihydropyrimidine dehydrogenase genomic DNA from the patient which comprises a residue at the position indicated as nucleotide 434 of SEQ ID NO: 1.

8. (Amended) The method of claim 7, wherein the method comprises amplifying the genomic DNA with a primer which hybridizes to a human dihydropyrimidine dehydrogenase genomic nucleotide sequence located within 500 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1.

9. (Amended) The method of claim 7, wherein the presence or absence of the G residue is detected by digestion of the amplified DNA with a restriction endonuclease.

10. (Amended) A composition comprising a PCR primer which binds to a human dihydropyrimidine dehydrogenase intronic genomic nucleotide sequence located within 500 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1.

11. (Amended) The composition of claim 10, wherein the PCR primer binds to a human dihydropyrimidine dehydrogenase intronic genomic nucleotide sequence located within 100 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1.

15. (Amended) A kit comprising a container, a first PCR primer which binds to DNA 3' of a splice site in the human genomic DNA for an exon encoding amino acids 581-635 of human dihydropyrimidine dehydrogenase, and a second PCR primer which binds to DNA 5' of a splice site in the human genomic DNA for an exon encoding amino acids 581-635 of human dihydropyrimidine dehydrogenase, wherein at least one of the first or second PCR primers binds to a human dihydropyrimidine dehydrogenase intronic genomic nucleotide sequence located within 500 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1.

16. (Amended) The kit of claim 15, wherein the kit further comprises instructions for the detection of the presence or absence of a G residue in human dihydropyrimidine dehydrogenase genomic DNA at the position indicated as nucleotide 434 of SEQ ID NO: 1.

17. (Amended) The kit of claim 15, wherein the kit further comprises a restriction endonuclease which recognizes a sequence comprising a residue in human

dihydropyrimidine dehydrogenase genomic DNA at the position indicated as nucleotide 434 of SEQ ID NO: 1.

20. (New) The method of claim 2, wherein the method comprises amplifying the genomic DNA with a primer which hybridizes to a human dihydropyrimidine dehydrogenase genomic nucleotide sequence located within 100 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1.

21. (New) The method of claim 4, wherein the restriction endonuclease recognizes a Mae II cleavage site.

22. (New) The method of claim 8, wherein the method comprises amplifying the genomic DNA with a primer which hybridizes to a human dihydropyrimidine dehydrogenase genomic nucleotide sequence located within 100 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1.

23. (New) The method of claim 9, wherein the restriction endonuclease recognizes a Mae II cleavage site.

24. (New) The kit of claim 15, wherein at least one of the first or second PCR primers binds to a human dihydropyrimidine dehydrogenase intronic genomic nucleotide sequence located within 100 nucleotides of the position indicated as nucleotide 434 of SEQ ID NO: 1.

25. (New) The kit of claim 17, wherein the restriction endonuclease recognizes a Mae II cleavage site.

26. (New) A kit comprising a container, a first PCR primer which binds to DNA 3' of a splice site in the human genomic DNA for an exon encoding amino acids 581-635 of human dihydropyrimidine dehydrogenase, a second PCR primer which binds to DNA 5' of a splice site in the human genomic DNA for an exon encoding amino acids 581-635 of human dihydropyrimidine dehydrogenase, and instructions for the detection of the presence or absence

of a G residue in human dihydropyrimidine dehydrogenase genomic DNA at the position indicated as nucleotide 434 of SEQ ID NO: 1.

27. (New) The kit of claim 26, wherein the kit further comprises a restriction endonuclease which recognizes a sequence comprising a residue in human dihydropyrimidine dehydrogenase genomic DNA at the position indicated as nucleotide 434 of SEQ ID NO: 1.

28. (New) The kit of claim 27, wherein the restriction endonuclease recognizes a Mae II cleavage site.

ABSTRACT OF THE DISCLOSURE

The present invention provides methods and compositions for detecting splicing defects in the dihydropyrimidine dehydrogenase gene. The methods and compositions are useful for identifying persons who are at risk of a toxic reaction to the commonly employed cancer chemotherapy agent, 5-fluorouracil.

a9